IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Barten, Piet Conf. No. 1890

Application No.: 10/553,301 Examiner: none assigned

Filed: August 1, 2006 Art Unit: 1638

For: **Brassica Plants with High Levels of Anticarcinogenic Glucosinolates**

Commissioner for Patents
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Third-Party Submission in Published Application Under 37 CFR §1.99

Requester submits herewith publications of which Requester is aware that Requester believes are material to the examination of Applicant's application.

This information disclosure statement is accompanied by Form PTO/SB/08 (substitute for Form PTO-1449) and legible copies of all non-patent literature listed in said form.

This submission is made within two months of the publication of the application by the United States Patent Office. The application was published on February 8, 2007, as publication no. 2007/0033675 A1.

However, the present US application was filed under 35 U.S.C. §371 from an international application having a filing date of April 13, 2004. The international application

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received application no. PCT/NL04/00244. The international application was published on October 21, 2004, as publication no. WO/2004/089065 A1.

Although Applicant designated the United States in his international application, Applicant also designated 95 other countries. Applicant also did not comply with the requirements for entering the United States under 35 U.S.C §371 until August 1, 2006, almost two years after the international application was published, and Requester could not have known that Applicant had entered a national application in the United States until February 8, 2007, more than two years after the international publication date.

Within two months of the publication of the international application, Requester could not reasonably have anticipated that Applicant would enter the United States and therefore afford Requester the opportunity to make the present submission to the United States Patent Office at that time.

Requester respectfully requests that the Examiner consider the accompanying materials. The appropriate fee as set forth at 37 C.F.R. §1.17(i) for processing this request is provided.

The person making this request and submission is a practitioner who signs below on the basis of the information supplied to him.

Pursuant to 37 C.F.R. §1.99(c), Requester provides proof of service on applicant below in accordance with 37 C.F.R. §1.248.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	10/553,301
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U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	U.S. Patent Document Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A1	US-			
	A2	US-			
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FOREIGN PATENT DOCUMENTS

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	C1	HANSEN, M., MOLLER, P. and SORENSEN, H.; Glucosinolates in Broccoli Stored Under Controlled Atmosphere; *J. Amer. Soc. Hort. Sci.*; June 1995; Pages 1069-1074; Volume 120, Number 6; American Society for Horticultural Science; Alexandria, VA, United States	
	C2	KUSHAD, M., BROWN, A., KURILICH, A., JUVIK, J., KLEIN, B., WALLIG, M., JEFFERY, E.; Variation of Glucosinolates in Vegetable Crops of *Brassica oleracea*; *J. Agric. Food Chem*; 1999; Pages 1541-1548; Volume 47; American Chemical Society; Washington, DC, United States	
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Glucosinolates in Broccoli Stored under Controlled Atmosphere

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Abstract. **Content of total and individual glucosinolates were determined in, 'Marathon' broccoli florets *(Brassica olerucea* L. var. *italica* stored 7 days at 10C under air, 0.5% O_2, 0.5% O_2+ 20% CO_2 or 20% CO_2 atmosphere, followed by transfer to air for 2 days. 'Marathon' broccoli contained glucoraphanin, glucobrassicin, neoglucobrassicin, glucoiberin, 4-methoxyglucobrassicin, progoitrin, glucoalyssin, and gluconasturtiin. The methylssulfinylalkylglucosinolates (glucoiberin and glucoraphanin) and the indol-3-ylmethylglucosinolates (glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin) accounted for 78% and 20% of the total content, respectively, in freshly harvested broccoli. CA treatment and storage time had no significant effect on the relative content of these two groups of glucosinolates. Freshly harvested broccoli contained 47 μmol glucosinolate/g dry weight. The total glucosinolate content increased 42% and 21% during 7 days storage under air and 0.5% O_2+ 20% CO_2, respectively, as compared to freshly harvested broccoli, and decreased 15% in broccoli stored under 20% CO_2. Treatment with 20% CO_2 in the absence of 0, resulted in visible CO_2 injury and water soaking of the tissue. Aeration had no significant effect on total glucosinolate content but reduced the glucobrassicin content 35% in broccoli stored 7 days under 0.5% O_2+ 20% CO_2 or 20% CO_2 atmosphere. In contrast, the 4-methoxyglucobrassicin content increased during storage under low O_2 atmosphere and increased further after transfer to air.**

Plants belonging to the order Capparales including Brassicaceae, are characterized by their content of glucosinolates (Bjerg and Sorensen, 1987a). Glucosinolates and their breakdown products are important aroma and flavor compounds in *Brassica* vegetables (MacLeod, 1976), such as cabbage, Brussels sprouts, broccoli, cauliflower, and horseradish. The most notable example is allyl isothiocyanate in mustard and horseradish arising from enzymic breakdown of sinigrin. This compound causes a pungent and lachrymatory response upon cutting and chewing (Gilbert and Nursten, 1972). Indol-3-ylmethylglucosinolates, which occur in appreciable amounts in several *Brassica* vegetables, are of interest for their potential contribution of anticarcinogenic compounds to the diet (Loft et al., 1992; McDanell et al., 1988).

Glucosinolates have a well defined structure with a side chain (R-group) and *D*- glucopyranose as β– thioglucoside attached to carbon atom no. 0 in (Z)-N-hydroximine sulfate esters (Table 1) (Olsen and Sorensen, 1981; Sorensen, 1990). The structural variation of the more than 100 glucosinolates isolated from various plant sources is mainly in the R-group (Fenwick and Heaney, 1983; Sørensen, 1990). This is also the case for glucosinolates identified as constituents of *Brassica* vegetables (Table 1).

Total and individual glucosinolate contents vary among cultivars and plant parts (Lewis and Fenwick, 198; Olsen and Sorensen, 1981; Sang et al., 1984; Rahman et al., 1986; VanEtten et al., 1976), but the concentration is also affected by nutrient level and cultivation practice (Heaney et al., 1983; Josefsson, 1970). During the plants growth and development, glucosinolates are synthesized from amino acids in a series of steps (Ettlinger and Kjær, 1968; Kjær, 1960; Kjær and Larsen, 1980; Underhill and Kirkland, 1980), where many details are still unknown (Bjerg et al., 1987; Sørensen, 1991).

At present, only limited information is available relating to glucosinolate metabolism in *Brassica* vegetables after harvest. Chong and Bérard (1983) reported the variation in glucosinolate breakdown products in three cabbage cultivars during refrigerated storage. They found that the concentration of the thiocyanate ion, volatile isothiocyanates, and goitrin declined during storage and this was associated with decreasing quality of the cabbage. Similar results were observed in cabbage stored under controlled atmosphere (CA), except that the cabbage had more volatile isothiocyanates and goitrin during the early storage period and the content declined at a higher rate towards the end of storage (Bérard and Chong, 1985). Others (Hansen, 1979; Toivonen et al., 1982) found that white cabbage stored under CA increased in pungency, mustiness, and bitterness, but they did not study changes in glucosinolate content.

Broccoli is a commodity that benefits from storage under increased CO_2 and reduced O_2 concentrations (Lipton and Harris, 1974; Makhlouf et al., 1989). Short term storage of broccoli under CA or in film wraps was found to extend shelf life and maintain quality by delaying yellowing and reducing loss of chlorophyll and ascorbic acid (Forney and Rij, 1991; Wang, 1979). It is not known to what extent increased CO_2 and reduced O_2 concentrations may affect glucosindlate content and thus flavor and nutritional quality of broccoli during storage. The objective of the present study was to determine the total and individual glucosinolates in broccoli stored under low O_2 and high CO_2 to understand better glucosinolate metabolism in *Brassica* vegetables after harvest.

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[2]Ministry of Agriculture and Fisheries, The Danish Institute of Plant and Soil Science, Dept. of Food Science and Technology, 5792 Aarslev, Denmark. To whom reprint requests should be addressed.
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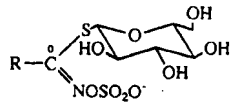
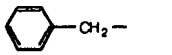
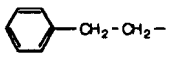
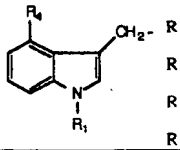
[4]Dept. of Vegetable Crops, Mann Laboratory.

Table 1. Numbers, structures and names of glucosinolates reported as constituents of *Brassica* vegetables[z].

Glucosinolate skeleton 

No	Structure of R-groups	Semisystematic names of R-groups[x]	Trivial names	Brassica spp.
1	$CH_2=CH-CH_2-$	Allyl	Sinigrin	Cabbage, Brussels sprouts, cauliflower, broccoli
2	$CH_2=CH-CH_2-CH_2-$	But-3-enyl	Gluconapin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
3	$CH_2=CH-CH_2-CH_2-CH_2-$	Pent-4-enyl	Glucobrassicanapin	Cauliflower, broccoli, Chinese cabbage
4	$CH_2=CH-\underset{OH}{CH}-CH_2-$	(2R)-2-Hydroxybut-3-enyl	Progoitrin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
7	$CH_3-S-CH_2-CH_2-CH_2-$	3-Methylthiopropyl	Glucoibervirin	Cabbage, cauliflower,
8	$CH_3-S-CH_2-CH_2-CH_2-CH_2-$	4-Methylthiobutyl	Glucoerucin	Cabbage, Brussels sprouts, cauliflower, broccoli
10	$CH_3-SO-CH_2-CH_2-CH_2-$	3-Methylsulphinylpropyl	Glucoiberin	Cabbage, Brussels sprouts, cauliflower, broccoli
11	$CH_3-SO-CH_2-CH_2-CH_2-CH_2-$	4-Methylsulphinylbutyl	Glucoraphanin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
12	$CH_3-SO-CH_2-CH_2-CH_2-CH_2-CH_2-$	5-Methylsulphinylpentyl	Glucoalyssin	Chinese cabbage
15	$CH_3-SO_2-CH_2-CH_2-CH_2-CH_2-$	4-Methylsulphonylbutyl	Glucoerysolin	Cabbage
16		Benzyl	Glucotropaeolin	Cabbage
17		Phenethyl	Gluconasturtiin	Cabbage, Brussels sprouts, broccoli, Chinese cabbage
23	 $R_1=H$ $R_4=H$	Indol-3-ylmethyl	Glucobrassicin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
24	$R_1=OCH_3$ $R_4=H$	N-Methoxyindol-3-ylmethyl	Neoglucobrassicin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
26	$R_1=H$ $R_4=OH$	4-Hydroxyindol-3-ylmethyl	4-Hydroxyglucobrassicin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage
27	$R_1=H$ $R_4=OCH_3$	4-Methoxyindol-3-ylmethyl	4-Methoxyglucobrassicin	Cabbage, Brussels sprouts, cauliflower, broccoli, Chinese cabbage

[z]Compiled from VanEtten et al. (1976), Heaney and Fenwick (1980), Lewis and Fenwick (1987,1988), Sones et al. (1984), Goodrich et al. (1989), and Lewis et al. (1991).
[x]The semisystematic names of glucosinolates are composed of the name of the R-group followed by the word glucosinolate, e.g., allylglucosinolate for number-1 (Sorensen, 1990 and references cited therin).

Materials and Methods

Plant material. 'Marathon' Broccoli heads *(Brassica oleracea* L. var. *italica)* were obtained on the day of harvest from Mann Packing Co., Salinas, Calif., top-iced and transported to the Mann Laboratory, Davis, Calif. where they were stored overnight at 0C. Miniflorets, 25 mm long and 20 to 70 mm in diameter, were excised from uniform heads of prime quality, surface sterilized in distilled water containing 100 ppm NaOCl for 5 min, drained, and divided on the basis of floret diameter into small (<40 mm), medium (40 to 50 mm) and large (>50 mm) sizes.

Storage under CA. Samples of broccoli (650 g) in a 1:2:1 (by weight) ratio of each floret size were placed in a 3.8-liter glass jar as one replicate, closed with a neoprene rubber stopper fitted with inlet and outlet polyethylene tubes. The jars were placed in a room at 10C for 7 days and ventilated with humidified gas at 10.1 ± 0.3 liters·h^{-1}. The atmospheres were as follows: air, 0.5% O_2, 0.5% O_2 + 20% CO_2, or 20% CO_2 (all balanced with N_2). After 7 days, the jars were transferred to air for 2 days at 10C. Oxygen and CO_2 concentrations were verified daily by analyzing 0.5 to 3 ml gas samples by electrochemical (model S-3All; Applied Electrochemical, Sunnyvale, Calif.) and infrared analyzers (model PIR-2000; Horiba, Irvine, Calif.). The variation in O_2 and CO_2 concentrations was within ± 5%. After 2, 7, and 9 days of storage, two samples per treatment were removed for analysis except from the treatment with 20% CO_2 atmosphere. In this treatment, samples were only removed at days 7 and 9.

Freeze-drying. Freshly harvested and stored broccoli florets (50 g) were frozen in liquid N_2 and kept in polyethylene bags at –40C until freeze-drying, usually within 1 month. Freeze-dried broccoli tissue was stored in sealed polyethylene bags at 4C until analysis.

Extraction and isolation of glucosinolates. Glucosinolates were extracted from freeze-dried, finely ground broccoli powder by the method of Bjerg et al. (1984). The samples (0.2 g) were spiked with a 100 µl internal standard solution containing 5.0 µmol·ml^{-1} of sinigrin and glucobarbarin, and extracted three times with 5 ml boiling 70% methanol for 2 min using an Ultra-Turrax Homogenizer (Ika-Labortechnik, Staufen, Germany). The extract obtained after centrifugation was concentrated to dryness *in vacuo,* and the residue was dissolved in 2 ml deionized water. Desulfoglucosinolates were prepared and quantitatively determined by HPLC according to Bjerg and Sorensen (1987b) and Sorensen (1990). The glucosinolate concentration was calculated using glucobarbarin as internal standard.

Statistical analysis. Statistical significance was assessed for total and individual glucosinolates by one-way and two-way ANOVA for unbalanced data (SAS, Cary, N.C.). The sources of variation were treatment (air, 0.5% O_2, 0.5% O_2 + 20% CO_2, and 20% CO_2) and time (0, 2, 7, and 9 days). Duncan's multiple range test and 95% confidence interval, respectively, were used to assess the location of the significant differences obtained.

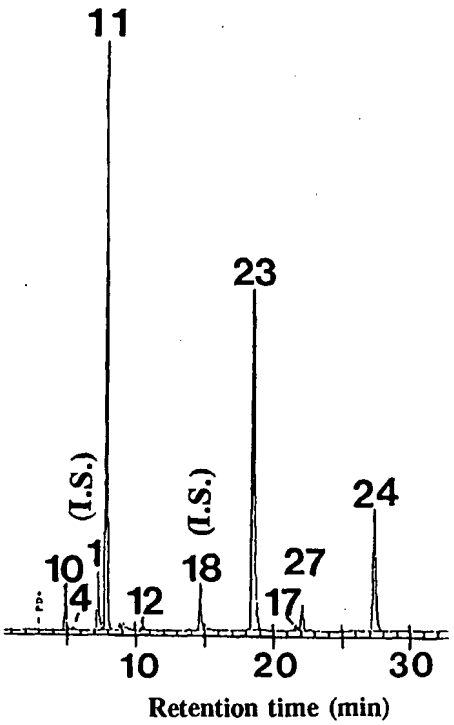


Fig. 1. Chromatogram of desulfoglucosinolates identified in freshly harvested 'Marathon' broccoli. The numbers refer to Table 1. No. 1 (sinigrin) and 18 (glucobarbarin) are internal standards (I.S.).

Results and Discussion

Total and individual glucosinolates. HPLC separation of individual desulfoglucosinolates from freshly harvested 'Marathon' broccoli is shown in Fig. 1. The broccoli contained glucoraphanin (11), glucobrassicin (23), neoglucobrassicin (24), glucoiberin (10), 4-methoxyglucobrassicin (27), progoitrin (4), glucoalyssin (12), and gluconasturtiin (17). The major glucosinolates (found in concentrations >1 $\mu mol \cdot g^{-1}$ dry weight) were glucoraphanin, glucobrassicin, glucoiberin, neoglucobrassicin, glucoiberin and 4-methoxyglucobrassicin. Others (Goodrich et al., 1989; Lewis et

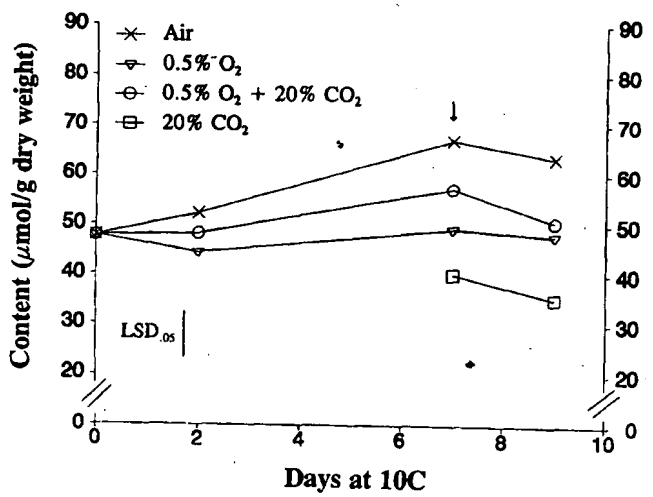


Fig. 2. Total glucosinolate content in broccoli stored 7 days under CA followed by 2 days aeration (⇓ transfer to air). Data are means of two replicates.

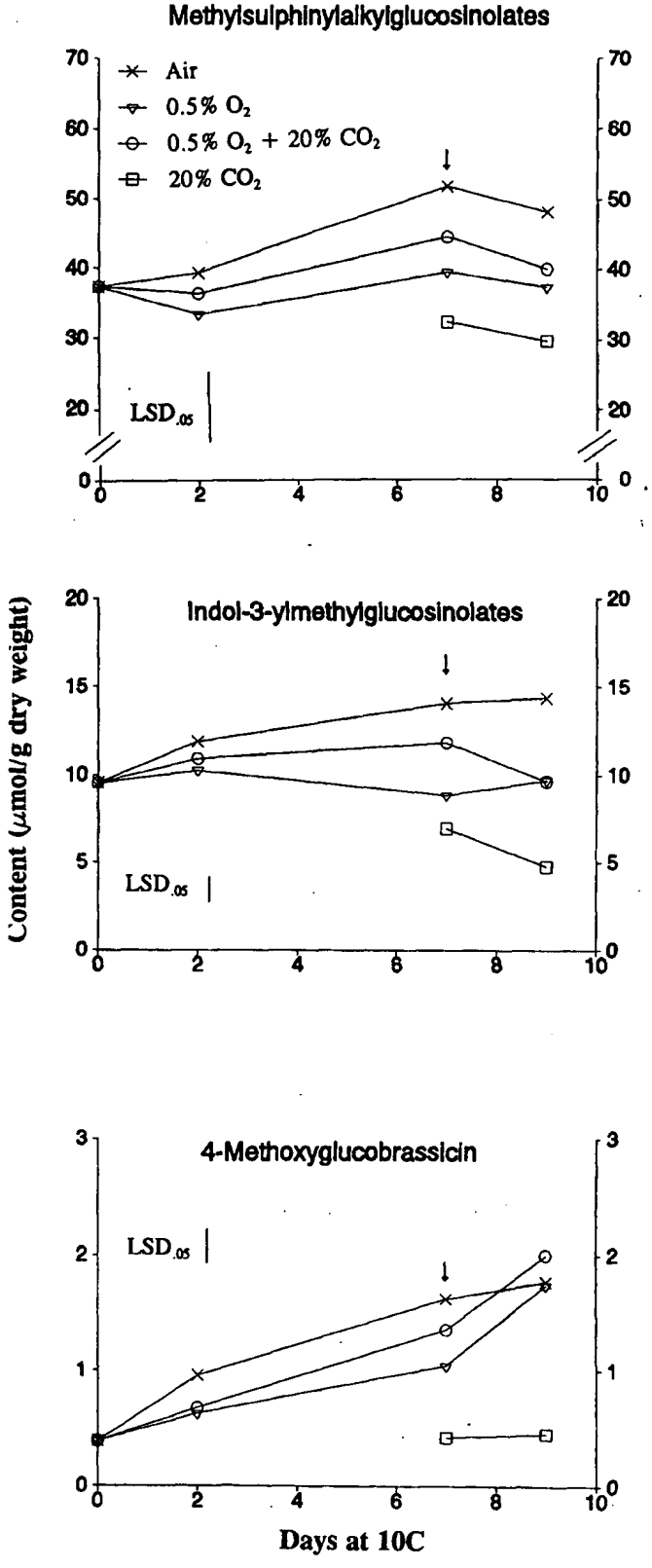


Fig. 3. Content of methylsulfinylalkylglucosinolates (glucoiberin and glucoraphanin), indol-3-ylmethylglucosinolates (glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin) and 4-methoxyglucobrassicin in broccoli stoned 7 days under CA followed by 2 days aeration (⇓ transfer to air). Data are means of 2 replicates.

Table 2. Average concentration of major glucosinolates (μmol·g^{-1} dry weight) in 'Marathon' broccoli stored 7 days under CA and transferred to air for 2 days. The relative content of the total is indicated in parentheses.

Glucosinolates [z]	Air	0.5% O_2	0.5% O_2 + 20% CO_2	20% CO_2
Glucoiberin	3.2 a[y](5)	2.5 bc (5)	2.8 ab (5)	2.0 c (5)
Glucoraphanin	46.6 a (71)	35.8 bc (73)	39.2 ab (72)	29.0 c (76)
Glucobrassicin [x]	10.6 a (16)	6.2 c (13)	7.3 b (13)	4.5 d (12)
Neoglucobrassicin	1.9 a (3)	1.7 a (3)	1.8 a (3)	0.9 b (2)
4-Methoxyglucobrassicin [x]	1.7 a (3)	1.4 b (3)	1.7 a (3)	0.4 c (1)
Methylsulphinylalkylglucosinolates [w]	49.8 c (76)	38.3 bc (79)	42.0 ab (78)	31.0 c (82)
Indol-3-ylmethylglucosinolates [v]	14.2 a (22)	9.3 b (19)	10.7 b (20)	5.8 c (15)
Total glucosinolates	65.5 a (100)	49.0 b (100)	54.2 b (100)	37.9 c (100)

[z]Chemical structures are shown in Table 1.
[y]Numbers within a row followed by different letters are significantly different at $P = 0.05$ by Duncan's multiple range test.
[x]Interaction between CA treatments and storage time.
[w]Methylsulphinylalkylglucosinolates: glucoiberin and glucoraphanin.
[v]Indol-3-ylmethylglucosinolates: glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin.

al., 1991) reported a similar glucosinolate profile in broccoli. Of the major glucosinolates, the methylsulfinylalkylglucosinolates (glucoiberin and glucoraphanin) and the indol-3-ylmethylglucosinolates (glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin) accounted for 78% and 20% of the total content, respectively, in freshly harvested broccoli.

Storage under air. Total glucosinolate content increased from 47.1 μmol·g^{-1} dry weight at day 0 to 67.0 μmol·g^{-1} dry weight at day 7 followed by a slight decline at day 9 as the broccoli deteriorated (Fig. 2). Incipient yellowing of florets was observed on day 7 and at day 9 the flower buds were moderate yellow (Hansen, 1993). Although the total glucosinolate content differed during air storage, none of the contents were significantly different from the others, probably due to too few observations. Chong and Bérard (1983) demonstrated that the concentration of glucosinolate products from cabbage increased during cold storage until the cabbage began to senescence, then the content rapidly declined. Analysis of variance for the content of individual glucosinolates indicated significant differences ($P = 0.05$) for 4-methoxyglucobrassicin. The content increased from 0.4 μmol·g^{-1} dry weight at day 0 to 1.8 μmol·g^{-1} dry weight at day 9 in air stored broccoli.

Storage under CA. The green color of the florets was maintained under the three CA conditions used. The 20% CO_2 atmosphere resulted in severe off-odors. The total glucosinolate content increased 42% and 21% during 7 days storage under air and 0.5% O_2 + 20% CO_2, respectively, as compared to freshly harvested broccoli (Fig. 2). This increase could have been associated with enhanced synthesis or a release of bound compounds during storage. Total glucosinolate 'content did not change for broccoli stored under 0.5% O_2, but decreased 15% in broccoli stored under 20% CO_2 in the absence of O_2. Exudation of cell sap, a symptom of physiological injury of the tissue, was visible in these latter samples. This symptom probably reflected membrane damage and cell rupture, conditions favorable for hydrolytic breakdown of glucosinolates by myrosinase catalyzed hydrolysis or autolysis (Olsen and Sorensen, 1981; Sorensen, 1990). In the intact cell, myrosinases are well separated from glucosinolates (Lüthy and Matile, 1984). When glucosinolates and myrosinases are brought in contact, a number of volatile and nonvolatile degradation products are formed depending on the structures of the glucosinolates and myrosinases and the actual conditions for hydrolysis (Sorensen, 1990; VanEtten and Tookey, 1983). During air and CA storage, the variation in the methylsulphinylalkylglucosinolate content (Fig. 3) was similar in both trend and magnitude to that of the total glucosinolates (Fig. 2). This result was in part due to the high relative content of methylsulphinylalkylglucosinolates (76% to 82%) in all samples (Table 2). The indol-3-ylmethylglucosinolate content (15% to 22% of total) increased 47% and 24% during 7 days storage under air and 0.5% O_2 + 20% CO_2 atmosphere, respectively, as compared to freshly harvested broccoli (Fig. 3). In contrast, the concentration did not change under 0.5% O_2 and decreased 28% following 7 days storage under 20% CO_2 (Fig. 3). No significant differences were found between the relative content of methylsulfinylalkyl- and indol-3-ylmethylglucosinolates with regard to CA treatment and storage period.

The average concentrations of total and individual glucosinolates for day 7 to 9 are shown in Table 2. Broccoli stored under air had the highest content of glucosinolates followed by that stored under 0.5% O_2 + 20% CO_2, 0.5% O_2, and 20% CO_2 (Table 2). There were

Table 3. Significance of CA treatments, storage time[z], and interactions for content of total and individual glucosinolates, methylsulphinylalkylglucosinolates[y] and, indol-3-yhnethylglucosinolates[x].

	Gluco-iberin	Gluco-raphanin	Gluco-brassicin	Neo-glucobrassicin	4-Methoxy-glucobrassicin	Methylsulphinyl-alkyl-glucosinolates	Indol-3-yl-methyl-glucosinolates	Total glucosinolates
CA-treatment	**	**	***	**	***	*	***	***
Storage time	NS	NS	**	NS	**	NS	NS	NS
Interaction	NS	NS	*	NS	*	NS	NS	NS

[z]Day 7 and 9.
[y]Methylsulphinylalkylglucosinolates: glucoiberin and glucoraphanin.
[x]Indol-3-ylmethylglucosinolates: glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin.
[NS]*,**,***Nonsignificant or significant at $P = 0.01$, 0.001 and 0.0001, respectively.

significant differences among CA-treatments in the content of total and individual glucosinolates for day 7 to 9 (Table 3).

When the broccoli stored under CA for 7 days was transferred to air for 2 days, there was not a significant decrease in total glucosinolate content (Table 3). Storage period (transfer to air from day 7 to 9) only affected glucobrassicin and 4-methoxyglucobrassicin contents. Aeration reduced the glucobrassicin contents 35% in broccoli stored 7 days under either 0.5% O_2 + 20% CO_2 or 20% CO_2. Treatment with 0.5% O_2 + 20% CO_2 probably caused physiological stress in the tissue even though no symptoms of CO_2 injury were visible. This could result in an increase in the hydrolytic breakdown of glucosinolates upon aeration. On average, the glucobrassicin content decreased from 7.8 $\mu mol \cdot g^{-1}$ dry weight at day 7 to 6.5 $\mu mol \cdot g^{-1}$ dry weight at day 9. The opposite result was noted for 4-methoxyglucobrassicin (Fig. 3). The content increased during storage under low 0, atmosphere and increased further after transfer to air. The average content of 4-methoxyglucobrassicin increased from 1.1 $\mu mol \cdot g^{-1}$ dry weight at day 7 to 1.5 $\mu mol \cdot g^{-1}$ dry weight at day 9. These results may indicate that storage could affect the nutritional value of broccoli since degradation products of indol-3-ylmethylglucosinolates, especially substituted indol-3-ylmethylglucosinolates, have been shown to have anticarcinogenic effects (Feldt et al., 1994 and references cited therein; Loft et al., 1992). In the present study, very low O_2 and very high CO_2 were imposed. Glucosinolate metabolism of broccoli stored at lower temperature and very extreme CA conditions should be investigated.

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Variation of Glucosinolates in Vegetable Crops of *Brassica oleracea*

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Glucosinolates were evaluated in 5 groups and 65 accessions of *Brassica oleracea* (50 broccoli, 4 Brussels sprouts, 6 cabbage, 3 cauliflower, and 2 kale) grown under uniform cultural conditions. Glucosinolates and their concentrations varied among the different groups and within each group. The predominant glucosinolates in broccoli were 4-methylsulfinylbutyl glucosinolate (glucoraphanin), 3-butenyl glucosinolate (gluconapin), and 3-indolylmethyl glucosinoate (glucobrassicin). Glucoraphanin concentration in broccoli ranged from 0.8 μmol g^{-1} DW in EV6-1 to 21.7 μmol g^{-1} DW in Brigadier. Concentrations of the other glucosinolates in broccoli varied similarly over a wide range. In Brussels sprouts, cabbage, cauliflower, and kale, the predominant glucosinolates were sinigrin (8.9, 7.8, 9.3, and 10.4 μmol g^{-1} DW, respectively) and glucobrassicin (3.2, 0.9, 1.3, and 1.2 μmol g^{-1} DW, respectively). Brussels sprouts also had significant amounts of gluconapin (6.9 μmol g^{-1} DW). Wide variations in glucosinolate content among genotypes suggest differences in their health-promoting properties and the opportunity for enhancement of their levels through genetic manipulation.

Keywords: *Glucosinolates; broccoli; Brassica oleracea; health; HPLC*

INTRODUCTION

Epidemiological data show that a diet rich in cruciferous vegetables, such as broccoli, cabbage, Brussels sprouts, cauliflower, and kale, can reduce the risk from a number of cancers and that the risk can be significantly reduced by an intake of as little as 10 g per day (Graham, 1983; Wattenberg, 1993; Kohlmeier and Su, 1997). The underlying mechanism(s) for the reduction of cancer by cruciferous vegetables is not clear. However, these vegetables are rich in sulfur-containing glucosides called glucosinolates. Glucosinolates are a class of more than 100 plant secondary metabolites present primarily in the crucifer family (Fenwick and Heaney, 1983). Glucosinolates exhibit minimal anticancer activity; however, upon cell damage they undergo hydrolysis by myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) to yield glucose, sulfate, and aglucones that can undergo fragmentation and/or molecular rearrangement yielding isothiocyanates, thiocyanates, oxazolindine-2-thiones, and nitriles, depending on the specific glucosinolate substrate, myrosinase isozyme, reaction pH, and presence of certain ions (Bones and Rossiter, 1996). A number of aglucone breakdown products have been reported to protect cells against cancer (Faulkner et al., 1998; Kohlmeier and Su, 1997; Tawfiq et al., 1995).

Several mechanisms have been proposed for cancer prevention by breakdown products of glucosinolates. There is substantial evidence linking ingestion of a glucosinolate rich diet with an increase in activity of phase II detoxification enzymes. Nijhoff et al. (1995) reported that consumption of cooked Brussels sprouts reduced colorectal cancer and enhanced the synthesis of the detoxification enzyme glutathione *S*-transferase. In murine hepa1c1c7 cells, glucosinolate breakdown products from cruciferous vegetables have been proposed to act as blocking agents against carcinogenesis by inducing quinone reductase activity (Tawfiq et al., 1995). The isothiocyanates, indole-3-carbinol (a product of glucobrassicin), phenylethylisothiocyanate (a product of gluconasturtiin), and sulforaphane (a product of glucoraphanin) have been shown to upregulate the synthesis of hepatic detoxification enzymes (Bradfield and Bjeldanes, 1984; Guo et al., 1992; Zhang et al., 1992) and protect against carcinogenesis (Morse et al., 1989; Wattenberg and Loub, 1978; Zhang et al., 1994). While most of the nitrile breakdown products have not been evaluated for their anticancer activity, a recent study demonstrated that the nitrile crambene (a product of progoitrin or epiprogoitrin) also upregulated the detoxification enzymes glutathione *S*-transferase and quinone reductase (Staack et al., 1998) and protects against aflatoxin-induced hepatocarcinogenesis in rats (Jeffery et al., 1996).

Comparative studies of glucosinolate distribution and variability between and within groups of the most widely consumed cruciferous vegetables such as broccoli, cabbage, cauliflower, Brussels sprouts, and kale are limited. To our knowledge, there are only two studies that have compared glucosinolate levels in the edible portions of more than three groups of these vegetables (Carlson et al., 1987b; Mullin and Sahasrabudhe, 1977). Even though one of these studies (Mullin and Sahasrabudhe, 1997) reports only total glucosinolates, the data suggest considerable differences in glucosinolate levels between and within the *Brassica* groups. In the more detailed study (Carlson et al., 1987a), 13 different glucosinolates have been reported in broccoli, Brussels sprouts, cauliflower, and kale. The authors reported that

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certain cultivars were highly correlated in their glucosinolate pattern and could be grouped together. Thus, broccoli with higher levels of glucoraphanin formed one group, Brussels sprouts and cauliflower with higher levels of glucobrassicin formed a second group, collard with higher levels of progoitrin formed a third group, and mustard green with higher levels of sinigrin formed a fourth group (Carlson et al., 1978a). However cabbage, an important *Brassica* vegetable, was not included in this study, and the glucosinolate comparisons were based on data collected over several years. This may confound the data, since in turnip, cabbage, and *Brassica napus* significant variations in glucosinolates are reported from year to year due to differences in climatic conditions (Carlson et al., 1987b; Fieldsend and Milford, 1994; Rosa et al., 1996).

Glucosinolate levels in *Brassica* are also affected by the growing location. Shelp and McLellan (1993) evaluated glucosinolate distribution in two broccoli cultivars grown at three different locations. They reported that differences in glucosinolate content among growing sites were greater than differences between cultivars. Factors that may have contributed to these differences included soil type, sulfate and nitrate fertilizer application, plant spacing, and date of harvest (Josefsson, 1970; Heany and Fenwick, 1980b; Griffiths et al., 1991; Rosa et al., 1996). Higher levels of glucosinolates were found in plants grown on clay soil than on sandy soil and in plants treated with sulfate than in plants treated with nitrate (Josefsson, 1970; Heaney and Fenwick, 1980b). In a study of *Brassica napus*, it was reported that higher nitrogen application increased the proportion of progoitrin at the expense of sinigrin (Zhao et al., 1994).

While previous studies on glucosinolate distribution in *Brassica* vegetables have indicated differences between and within groups, a significant portion of the variation may have resulted from variations in environmental and cultural factors. Therefore, the objective of this study was to compare glucosinolate levels in the edible portions of 66 genotypes from five *Brassica oleracea* groups including 50 var. italica (broccoli), 4 var. gemmifera (Brussels sprouts), 6 var. capitata (cabbage), 3 var. butrytis (cauliflower), and 2 acephala (kale) grown under uniform cultural practices in an attempt to identify differences due to genetic factors. Emphasis was placed on broccoli because of its economic importance and consumer preference.

MATERIALS AND METHODS

Materials. Broccoli, Brussels sprouts, cabbage, cauliflower, and kale seeds were obtained from the Asgrow Seed Co. and Peto Seeds (Seminis vegetable seeds), the USDA Plant Genetics Resource Unit (Geneva, NY), and Dr. Mark Farnham of the USDA Vegetable Research Center (Charleston, SC). Lines included commercial hybrids, open-pollinated varieties, land races, inbreds, and doubled haploids developed by Farnham (1997) from commercial hybrids. In July 1996, seeds were germinated in Fafard growing mix no. 2 (Conrad Fafard, New Brunswick, Canada) in a greenhouse under natural day light. After 4 weeks in the greenhouse, seedlings were transplanted into a field plot of silty clay loam (Drumer) soil (pH 6.7) at the University of Illinois vegetable farm, in Champaign, IL. The field design was a randomized complete block (RCB) with three replicates of 15 plants each. Transplants of each entry were planted in single rows 75 cm apart and 40 cm in the row for a population equivalent of 33 333 plants per hectare. Water, fertilizer, and pesticides were applied according to standard cultural practices developed for cruciferous vegetables in Illinois (Foster and Maynard, 1998).

At optimum maturity, three uniform size plants free of insect and/or mechanical damage were selected from each replicate and the edible portions were cut, placed on ice, and immediately transported to the laboratory. Subsamples of 100 g from each plant per replicate were combined, weighed, frozen in liquid nitrogen, and lyophilized. Freeze-dried tissue was ground into a fine powder using a coffee mill and stored at −20 °C until further analysis.

Glucosinolates Analysis. Intact glucosinolates were analyzed according to Wathelet et al. (1991), with slight modification. A 0.2 g sample of freeze-dried powder from each replicate was placed in a capped 15 mL glass tube and heated on a heating block (Reacti-Therm III, Pierce, Rockford, IL) set at 95 °C for 15 min. To each tube, 2 mL of boiling deionized water and 500 μL of 1 μM benzylglucosinolate (internal standard, Canola Council of Canada, Manitoba, Canada) were added. The tubes were heated for an additional 5 min, immediately cooled on ice, and centrifuged at 12000*g* for 10 min at 4 °C, and the supernatant was saved on ice. The pellet was re-extracted with 1 mL of boiling water and centrifuged at 12000*g* for 10 min at 4 °C, and the supernatant was collected, combined with the previously saved supernatant, and mixed. A 1 mL fraction of the supernatant was combined with 150 μL of 0.5 M barium acetate, vortexed for 5 s, and layered on a DEAE Sephadex A-25 column. Glucosinolates were desulfated with arylsulfatase while on the column by adding 10 units of sulfatase suspended in 500 μL of glass-distilled water to each column and capping it for 18 h. The desulfated glucosinolates were eluted from the column with 2 mL of water and separated on a Hitachi HPLC system (Hitachi Ltd., Tokyo, Japan) consisting of a variable UV detector set at 229 nm wavelength, a refrigerated autosampler, a column heater set at 32 °C, and a Lichosphere RP-18 column (Merck, Darmstadt, Germany). Desulfoglucosinolates were eluted off the column in 46 min with a linear gradient of 0−20% acetonitrile in water at a flow rate of 0.8 mL/min. The type and amount of glucosinolates in each *Brassica* vegetable were calculated in comparison to certified glucosinolate levels in a standard rapeseed reference material (BCR 367, Commission of the European Community Bureau of References, Brussels, Belgium). Using benzylglucosinolate as an internal standard, the recovery of glucosinolates from the *Brassica* vegetables using this procedure was estimated at 95−97%. A representative chromatogram of the HPLC elution profile of glucosinolates from a broccoli sample is shown in Figure 1.

Statistical Analysis. Analysis of variance was performed on data between and within the groups. Mean separation was determined by least significant differences (LSD) at $P = 0.05$ and standard error of the mean (SE). Mean separation was also determined for the effect of harvest date on glucosinolates in broccoli. Pearson's correlations of glucosinolates and vitamins evaluated in a companion study by Kurilich et al. (1998) were also determined.

RESULTS AND DISCUSSION

Variation in Glucosinolates among *Brassica oleracea* Groups. Intact glucosinolates in broccoli, Brussel sprouts, cabbage, cauliflower, and kale were evaluated in order to determine variation in amounts and types across groups grown under similar conditions and using the same analytical procedure. A profile of 12 glucosinolates in *Brassica oleracea* groups is shown in Table 1. Data show significant differences in content of individual glucosinolate levels among the groups. In broccoli, the predominant glucosinolates were glucoraphanin (7.1 μmol g^{-1} DW), glucobrassicin (1.1 μmol g^{-1} DW), gluconapin (1.0 μmol g^{-1} DW), and progoitrin (1.0 μmol g^{-1} DW). Glucoraphanin and glucobrassicin concentrations in broccoli in this study were similar to those previously reported by Shelp et al. (1993) and Goodrich et al. (1989), but nearly 4-fold less than those reported by Hansen et al. (1995). Using gas chromatography,

Table 1. Mean Glucosinolate Content (μmol g^{-1} Dry Mass) in the Edible Tissues of *Brassica oleracea*[a]

glucosinolates						
group	form	broccoli	Brussels sprouts	cabbage	cauliflower	kale
aliphatics	sinigrin	0.1 ± 0.4	8.9 ± 0.2	7.8 ± 0.1	9.3 ± 0.1	10.4 ± 0.0
	gluconapin	1.0 ± 1.5	6.9 ± 0.7	0.7 ± 0.4	0.3 ± 0.2	1.0 ± 0.1
	glucobrassicanapin	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	progoitrin	1.0 ± 0.8	2.4 ± 0.4	0.2 ± 0.2	0.3 ± 0.1	0.6 ± 0.1
	epiprogoitrin	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	napoleiferin	0.7 ± 0.6	0.4 ± 0.3	0.0 ± 0.1	0.2 ± 0.1	0.0 ± 0.0
	glucoiberin	0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	glucoraphanin	7.1 ± 2.5	1.0 ± 1.3	0.1 ± 0.6	0.5 ± 0.3	1.0 ± 0.2
	glucoalysin	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
phenyl indoles	gluconasturtiin	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
	glucobrassicin	1.1 ± 0.4	3.2 ± 0.2	0.9 ± 0.1	1.3 ± 0.1	1.2 ± 0.0
	4-OH glucobrassicin	0.2 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	1.6 ± 0.0	0.1 ± 0.0
	4-CH_3OH glucobrassicin	0.4 ± 0.4	0.4 ± 0.2	0.3 ± 0.1	1.0 ± 0.1	0.2 ± 0.0
	neoglucobrassicin	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
	total	12.8	25.1	10.9	15.1	15.0

[a] Values represent the mean ± SEM. Means and SEM were calculated from the following accessions: broccoli, 50; Brussels sprouts, 4; cabbage, 6; cauliflower, 3; kale, 2.

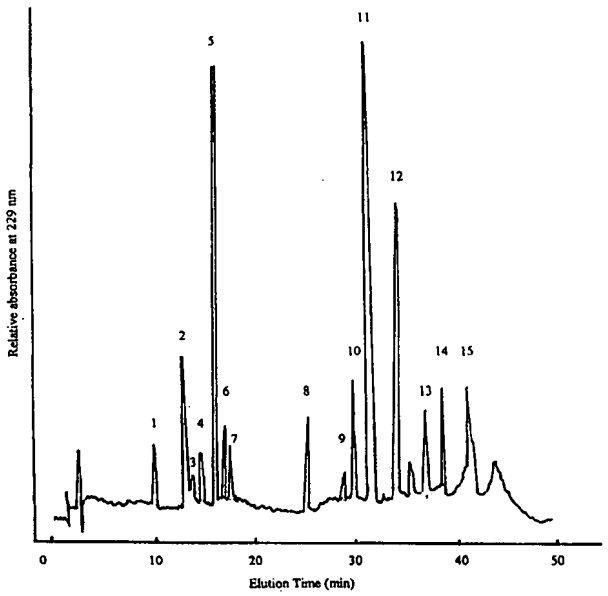

Figure 1. Typical HPLC chromatogram of desulfoglucosinolates in broccoli. Peaks: 1, glucoiberin; 2, progoitrin; 3, epiprogoitrin; 4, sinigrin; 5, glucoraphanin; 6, napoleiferin; 7, glucoalysin; 8, gluconapin; 9, 4-hydroxy-glucobrassicin; 10, glucobrassicanapin; 11, benzylglucosinolate (internal standard); 12, glucobrassicin; 13, gluconasturtiin; 14, 4-methoxy-glucobrassicin; 15, neoglucobrassicin.

Carlson et al. (1987b) found that in broccoli, the concentrations of glucoraphanin and glucobrassicin were similar. However, in the present study we found glucoraphanin concentration to be about 7-fold higher than that of glucobrassicin (Table 1). It is interesting to note that while estimation of glucoraphanin and glucobrassicin levels in the previous studies were made in mature flower heads, a more recent study (Fahey et al., 1997) reported a complete absence of glucobrassicin in broccoli seedlings. Similarly, we found 50% higher glucobrassicin in fully developed Packman broccoli heads (15−20 cm diameter) than in immature heads (5−10 cm diameter, data not shown). These results suggest that glucobrasccin synthesis is active during later stages of broccoli development. In Brussels sprouts, the predominant glucosinolates are sinigrin (8.9 μmol g^{-1} DW), gluconapin (6.9 μmol g^{-1} DW), glucobrassicin (3.2 μmol g^{-1} DW), and progoitrin (2.4 μmol g^{-1} DW). Our results are similar to those previously reported by Heaney and Fenwick (1980b) and Goodrich et al. (1989). In contrast, Carlson et al. (1987a) reported a higher level of glucobrassicin in Brussels sprouts than that reported in the present study and that the glucobrassicin concentration is nearly 40-fold higher than the sinigrin concentration. However, Carlson et al. (1987a) quantified glucobrassicin indirectly by measuring the amount of thiocyanate ion released by the action of myrosinase. This procedure appears to overestimate glucobrassicin concentration and does not differentiate between glucobrassicin and neoglucobrassicin (Heaney and Fenwick, 1980a). In a more recent study, Heaney and Fenwick (1993) compared several glucosinolate analysis procedures and suggested that high-pressure liquid chromatography is more reliable and less time-consuming than other methods.

In cabbage, the predominant glucosinolates were sinigrin (7.8 μmol g^{-1} DW), glucobrassicin (0.9 μmol g^{-1} DW), and gluconapin (0.7 μmol g^{-1} DW). In agreement with our data, Pocock et al. (1987) found high levels of sinigrin and glucobrassicin compared to the other glucosinolates in English white cabbage. In addition, they found significant amounts of glucoiberin in the three cultivars tested. In a survey of 22 cabbage cultivars, VanEtten and Daxenbichler (1976) found higher levels (10−50%) of glucoiberin than either sinigrin or glucobrassicin. Rosa (1997) reported that glucoiberin concentration in cabbage is about 2-fold higher in the roots than in the leaves and it decreased in both tissues with an increase in plant age. In the present study, glucoiberin concentration was very low (≤0.06 μmol g^{-1} DW) in the six accessions tested. Both Pocock et al. (1987) and VanEtten and Daxenbichler (1976) used GC to detect glucoiberin, which suggests that GC may be more sensitive than HPLC for detecting this compound.

In cauliflower, the most abundant glucosinolates were sinigrin (9.3 μmol g^{-1} DW), 4-hydroxy glucobrassicin (1.6 μmol g^{-1} DW), and glucobrassicin (1.3 μmol g^{-1} DW). Carlson et al. (1987b) and Sones et al. (1984) also found higher levels of sinigrin and glucobrassicin compared to other glucosinolates in cauliflower. However, the amounts that they reported are significantly less than those we report in this study. Both Carlson et al. (1987b) and Sones et al. (1984) used GC to estimate the level of glucosinolates. According to Sones et al. (1984), the GC

procedure underestimates the level of glucosinolates in cauliflower.

In kale, the most abundant glucosinolates were sinigrin (10.4 μmol g^{-1} DW), glucobrassicin (1.2 μmol g^{-1} DW), gluconapin (1.0 μmol g^{-1} DW), and glucoraphanin (1.0 μmol g^{-1} DW, Table 1). Similarly, Carlson et al. (1987a) and Rosa et al. (1996) reported higher sinigrin and glucobrassicin but only a trace amount of glucoraphanin in kale leaves. However, in flowering Chinese kale, up to 68% of the total glucosinolates were reported to be glucoraphanin (Hill et al., 1987). In this study, gluconasturtiin concentration in kale was similar to that in the other four vegetable groups (Table 2).

An important factor that may have contributed to the difference in glucosinolate content among the groups is the difference in tissue types tested. In this study, immature flowers were analyzed in broccoli and cauliflower, leaves in cabbage and kale, and axillary buds in Brussels sprouts. Variation in glucosinolate types and concentrations among plant parts was previously reported in turnip, cabbage, and *Brassica napus* (Booth and Walker, 1991; Carlson et al., 1987a; Clossais-Besnard and Larher, 1991; Rosa, 1997). In turnip, gluconapin and glucobrassicanapin were higher in the tops than in the roots, while glucoraphanin, progoitrin, glucoalysin, glucobrassicin, and total glucosinolates were higher in the roots than in the tops (Carlson et al., 1987a; Rosa et al., 1996). In cabbage, Rosa (1997) found higher indole glucosinolates in the roots and higher aliphatic glucosinolates in the leaves. Differences in glucosinolate levels between plant parts are not linked to mobilization between organs; instead they are believed to be synthesized and stored within each organ (Rosa et al., 1996; Clossais-Besnard and Larher, 1991).

Variation in Glucosinolates within *Brassica oleracea* Groups. Evaluation of glucosinolate levels showed significant differences between accessions within each group (Table 2). Among the broccoli accessions tested, the highest amount of glucoraphanin was found in Brigadier (21.7 μmol g^{-1} DW followed by Wintergarden (17.5 μmol g^{-1} DW), and Majestic (16.0 μmol g^{-1} DW). The lowest amount of glucoraphanin (0.8 μmol g^{-1} DW) was found in EV6-1. Carlson et al. (1987b) also found differences in glucoraphanin among six broccoli accessions; however, the differences were relatively small (less than 3-fold) compared to differences reported in this study (more than 27-fold). In *Brassica napus* and *Brassica oleracea*, Rosa (1996) reported a significant effect of accessions on glucosinolate levels.

In some broccoli accessions (example Peto no. 3), the glucoraphanin level represents up to 90% of the aliphatic glucosinolates. The action of the hydrolyzing enzyme myrosinase on glucoraphanin causes the release of glucose and an unstable intermediate that rearranges irreversibly to form sulforaphane and/or sulforaphane nitrile. Basic pH and high temperature enhance sulforaphane formation, while acidic pH and ferrous ions enhance sulforaphane nitrile formation (Fenwick et al., 1983). Sulforaphane is the most potent naturally occurring inducer of phase II enzymes, including quinone reductase and glutathione *S*-transferases (Prochaska et al., 1992). Induction of phase II enzymes has been shown to protect cells against toxic electrophiles, including carcinogens such as 9,10-dimethyl-1,2-benzanthracene (Zhang et al., 1992, 1994). Sulforaphane nitrile, however, is inactive against hepatic glutathione concentration and glutathione-*S*-transferase activity (Ringenberg and Wallig, 1996). In broccoli, the concentration of sulforaphane was reported to be between 1.9 and 3.7 mg g^{-1} DW and sulforaphane nitrile between 0.2 and 0.3 mg g^{-1} DW (Chiang et al., 1998).

Other aliphatic glucosinolates found in varying amounts in broccoli include epiprogoitrin, napoleiferin, glucoalysin, and gluconapin. The sum of these compounds is listed as residual aliphatics (Table 1). Differences in concentrations of these compounds among the broccoli accessions were relatively small, except in DeCicco, which showed significantly higher concentration of glucoalysin (16.0 μmol g^{-1} DW), and Peto no. 7, which showed significantly higher concentrations of progoitrin (7.9 μmol g^{-1} DW) and gluconapin (17.1 μmol g^{-1} DW). Differences in the profile of aliphatic glucosinolates among broccoli lines have been attributed to genetic variation (Giamoustaris and Mithen, 1996). Several loci have been identified that are involved in the partitioning of aliphatic glucosinolates. The *Gsl-elong* locus is responsible for the elongation of the carbon side chain (Mithen et al., 1995), while several other loci are affiliated with side chain rearrangements (Giamoustaris and Mithen, 1996; Magrath et al., 1994). Partitioning into the various forms has been proposed to be regulated by the presence of nonfunctional alleles at these loci (Giamoustaris and Mithen, 1996).

Broccoli accessions also showed significant differences in indole glucosinolates content. Glucobrassicin is the most abundant indole glucosinolate in broccoli, representing nearly 60% of the indoles and about 10% of the total glucosinolates (Table 2). The highest concentration of glucobrassicin in broccoli was found in the accessions Pirate (2.8 μmol g^{-1} DW) and Shogun (2.4 μmol g^{-1} DW), while the lowest concentration was found in Galaxy, Majestic, and MA 191 DH X6 (0.3 μmol g^{-1} DW). The sums of residual indole glucosinolates (4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin) were small and relatively similar among the broccoli accessions (Table 2), except in Peto no. 10 which contained a significant amount of 4-hydroxy glucobrassicin (3.4 μmol g^{-1} DW). In contrast to aliphatic glucosinolates, which are predominately under genetic control (Magrath et al., 1994), the concentration of the indole forms has been proposed to be regulated primarily by environmental and/or physiological factors (Mithen et al., 1995).

In Brussels sprouts, the accession Jersey had nearly 2−3-fold higher aliphatic glucosinolates than the other four accessions (Table 2). The predominant aliphatic glucosinolates in Jersey were gluconapin, sinigrin, and progoitrin at 12.5, 9.1, and 7.3 μmol g^{-1} DW, respectively. Significant differences were also detected in indole glucosinolates content among the five Brussels sprouts accessions (Table 2). The accessions Cambridge no. 5 and Yates Darkcrop have 56 and 69% higher glucobrassicin concentration than the accession Long Island, respectively. Other indole glucosinolates (summed as residual) were relatively similar among the Brussels sprouts accessions (Table 2).

Among cabbage accessions, PI 1214148 had the highest concentration of total aliphatic glucosinolates (39.0 μmol g^{-1} DW), while Peto no. 27 had the lowest (5.6 μmol g^{-1} DW) concentration. The predominant aliphatic glucosinolates in PI 1214148 were sinigrin (21.1 μmol g^{-1} DW), gluconapin (13.4 μmol g^{-1} DW, included in the residual aliphatic), and progoitrin (2.3 μmol g^{-1} DW).

Table 2. Glucosinolates Content (μmol g^{-1} DW) of Individual Accessions within Five *Brassica oleracea* Subspecies

	aliphatics					indoles			phenyl
accession	sinigrin	progoitrin	glucoraphanin	residual	total	glucobrassicin	residual	total	gluconasturtiin
Broccoli									
Atlantic	0.0	0.3	11.6	1.1	12.9	0.7	0.6	1.4	0.5
Baccus	0.0	0.7	1.5	1.2	3.4	0.4	0.7	1.0	0.1
Big Sur	0.0	0.2	2.2	0.6	3.0	0.4	1.0	1.4	0.4
Brigidier	0.0	0.9	21.7	3.8	26.3	1.2	1.0	2.2	0.7
Cavalo Broccolo Ramoso	0.2	1.4	10.2	6.2	18.0	1.8	3.9	5.7	0.8
De Cicco	0.0	0.4	10.7	2.7	13.8	1.7	1.0	2.8	0.6
Eu 4-1	0.0	0.2	7.6	0.4	8.2	0.9	0.6	1.5	0.3
Eu 8-1	0.1	7.9	9.6	3.3	20.9	1.3	1.0	2.3	0.6
EV 2-1	0.0	0.2	1.7	1.1	3.0	1.5	1.3	2.8	0.8
EV 6-1	0.0	0.1	0.8	0.4	1.3	0.6	0.5	1.1	0.2
Florette	0.0	0.3	8.7	1.5	10.4	1.0	0.5	1.6	0.6
Fu 549 DH I.S.	0.0	0.3	3.9	3.9	8.1	0.7	0.3	1.1	0.4
G31824	0.0	0.5	7.1	2.5	10.1	0.5	1.6	2.1	0.8
G31825	0.0	3.9	3.3	4.4	11.6	0.6	0.2	0.8	0.2
Galaxy	0.0	1.0	3.4	0.7	5.1	0.3	0.2	0.5	0.2
GC 1-2	0.0	0.1	5.1	0.5	5.7	0.7	0.8	1.4	0.2
GC 3-2	0.3	2.1	1.6	3.2	7.2	1.3	0.2	1.5	0.2
Gem	0.0	0.0	5.0	1.3	6.4	0.5	0.3	0.7	0.3
Green Comet	0.0	1.4	6.4	1.4	9.2	0.4	0.4	0.9	0.3
Greenbelt	0.0	0.4	8.5	0.9	9.8	1.2	0.7	1.8	0.3
GV 8-1	0.0	2.0	6.6	3.9	12.4	0.7	0.9	1.6	0.3
High Sierra	0.3	2.3	4.5	2.9	9.9	0.8	0.6	1.3	0.3
HS 061 DH I.S.	0.0	0.2	7.2	1.1	8.5	0.8	0.4	1.2	0.3
HS 067 DH I.S.	0.0	0.3	7.4	0.7	8.4	0.8	0.4	1.2	0.3
Legacy	0.0	0.1	5.5	1.9	7.5	0.4	0.4	0.8	0.4
MA 065 DH I.S.	0.0	0.2	6.2	0.6	7.0	1.6	0.7	2.4	0.4
MA 191 DH X6	0.1	0.0	13.8	3.8	17.7	0.3	0.7	1.1	0.4
Majestic	0.0	0.1	16.0	3.0	19.1	0.3	0.4	0.7	0.2
Packman	0.1	0.9	4.2	2.4	7.5	0.1	0.3	0.4	0.1
Persius	0.0	0.5	10.6	0.8	11.9	1.0	0.5	1.6	0.5
Peto no. 3	0.0	0.2	7.1	0.6	7.8	0.5	0.3	0.8	0.1
Peto no. 5	0.0	0.3	8.8	0.9	10.1	1.1	0.5	1.5	0.3
Peto no. 6	0.0	0.0	5.5	1.3	6.8	1.5	0.9	2.3	0.4
Peto no. 7	0.1	7.2	5.9	18.3	31.4	1.2	2.4	3.6	0.6
Peto no. 8	0.0	0.1	5.1	0.5	5.8	0.9	0.5	1.4	0.4
Peto no. 10	0.0	0.4	8.5	1.4	10.3	1.8	4.2	6.0	0.6
Peto no. 11	0.0	0.9	2.6	1.4	4.9	1.0	0.8	1.8	0.2
Peto no. 12	0.1	0.1	3.6	4.3	8.0	0.3	0.5	0.8	0.1
Peto no. 13	0.0	0.3	6.0	1.1	7.4	1.0	0.4	1.4	0.4
Peto no. 14	0.0	0.1	7.6	0.9	8.6	0.7	0.5	1.2	0.2
Peto no. 15	0.0	0.6	2.2	0.9	3.7	0.7	0.3	1.0	0.2
Peto no. 16	0.0	0.2	9.1	1.0	10.2	1.5	0.4	1.9	0.4
Pinnacle	0.1	0.3	8.8	2.2	11.4	1.8	1.9	3.7	0.7
Pirate F1	0.0	0.5	10.7	1.9	13.0	2.8	1.3	4.1	0.6
Premium Crop	0.0	1.1	4.7	2.4	8.2	1.3	0.8	2.1	0.4
Shogun	0.8	2.6	11.9	3.3	18.7	2.4	3.8	6.2	0.9
Su 006 DH I.S.	0.2	0.4	1.5	8.7	10.7	1.8	0.8	2.7	0.4
VI 158 DH I.S.	6.0	5.3	9.4	10.5	31.2	1.3	0.6	1.9	0.5
Wintergarden	0.0	0.5	17.5	0.9	18.9	1.6	0.9	2.5	0.5
Zeus	0.0	0.1	2.9	0.8	3.8	1.1	1.3	2.3	0.3
Brussels Sprouts									
Cambridge no. 5	3.2	1.9	0.6	6.2	11.9	5.6	1.6	7.1	0.6
Jersey	9.1	7.3	1.3	14.1	31.7	3.9	0.8	4.6	0.6
Long Island	4.6	1.1	0.4	5.5	11.5	3.6	1.3	4.9	0.2
Yates Darkcrop	5.1	2.2	1.4	6.1	14.8	6.1	1.8	7.9	0.6
Cabbage									
Jersey Wakfield	6.9	0.6	0.4	2.5	10.5	0.6	1.0	1.5	0.4
Peto no. 22	11.2	0.4	0.3	1.2	13.1	0.6	0.4	1.0	0.4
Peto no. 23	7.1	0.2	0.1	0.1	7.4	0.5	0.5	1.0	0.2
Peto no. 24	7.4	0.2	0.1	0.8	8.5	1.3	1.0	2.3	0.6
Peto no. 27	4.3	0.2	0.1	1.0	5.6	1.3	1.0	2.3	0.2
PI 1214148	21.1	2.3	1.8	13.8	39.0	0.9	0.6	1.5	0.7
Cauliflower									
Feng Shan	9.5	0.3	0.2	0.4	10.3	1.1	1.7	2.8	0.6
Peto no. 17	5.7	0.3	0.4	0.8	7.1	0.3	2.4	2.7	0.2
Snow Crown	12.9	0.3	0.9	0.6	14.6	2.3	3.8	6.1	0.4
Kale									
Vates	7.4	0.5	0.6	1.1	9.5	1.6	0.5	2.2	0.4
Winterborne	13.3	0.7	1.4	1.2	16.6	0.7	0.3	1.0	0.4
LSD 0.01 (all)	3.6	1.4	6.3	0.4	11.0	1.8	1.2	3.0	0.5
LSD 0.01 (broccoli only)	1.0	1.3	7.2	0.1	9.7	1.5	1.1	2.7	0.5

[a] Values represent the mean of three replicates per accession.

Concentrations of indole glucosinolates were relatively similar among the cabbage accessions tested.

In cauliflower, sinigrin concentration varied among accessions (Table 2). The highest concentration of sinigrin was found in Snow Crown (12.9 μmol g^{-1} DW) while the lowest concentration was found in Peto no. 17 (5.7 μmol g^{-1} DW). Similarly, Snow Crown showed a significantly higher concentration of indole glucosinolates, especially of glucobrassicin (2.3 μmol g^{-1} DW) and 4-hydroxy glucobrassicin (3.4 μmol g^{-1} DW, included in residual indoles), than Peto no. 17 and Feng Shan. Peto no. 17 showed a significantly higher concentration of methoxy glucobrassicin (1.8 μmol g^{-1} DW) compared to Snow Crown and Feng Shan (0.3 and 0.7 μmol g^{-1} DW, respectively). No major differences in the other indole glucosinolates were observed among the three cauliflower accessions.

In kale, the accession Winterborne had about 2-fold higher total aliphatic glucosinolates content (predominantly sinigrin) than the accession Vates. However, Vates had about 2-fold higher total indole glucosinolates (predominantly glucobrassicin) than Winterborne (Table 2).

In addition to genetic factors, variation in glucosinolate content within the groups of *Brassica* examined in this study may have resulted from differences in dates of harvest. In broccoli, we have found a positive and significant correlation ($r = 0.52$, $P = 0.004$) between glucoraphanin levels and date of harvest. For example, the late maturing accessions Peto no. 5 and Peto no. 10 (maturing 79 and 85 days after transplanting, respectively) contained 8.84 and 8.50 μmol g^{-1} DW glucoraphanin, while the earlier maturing accessions EV6-1 and Peto no. 11 (both maturing at 55 days after transplanting) contained 0.8 and 2.6 μmol g^{-1} DW glucoraphanin, respectively (Table 2). Glucoraphanin levels drop precipitously during the first 10 days of growth from seed (Fahey et al., 1997). Rosa et al. (1996) reported higher glucosinolates in spring than fall harvested cabbage. However, these differences were not linked to variation in growing temperatures (Rosa, 1997). In contrast, in a companion study we have found no correlation between broccoli harvest date and carotenoids, tocopherols, and ascorbate levels (Kurilich et al., 1998).

Interaction between Glucosinolates and Vitamins. In a companion study, using the same cruciferous accessions, we have evaluated the levels of the antioxidants α- and γ-tocopherols, α- and β-carotenes, and ascorbate (Kurilich et al., 1998). Pearson's correlations between glucosinolates and antioxidants in broccoli showed that there are significant and positive interactions between glucoraphanin and α-tocopherol ($r = 0.30$, $P = 0.02$). Significant and positive correlations were also found between total aliphatic glucosinolates and γ-tocopherol ($r = 0.38$, $P = 0.006$) and gluconasturtiin and γ-tocopherol ($r = 0.28$, $P = 0.05$). However, there were no significant correlations between indole glucosinolates and any of the antioxidants (Kurilich et al., 1998). Vang et al. (1997) reported that anticarcinogenic properties of dietary fruit and vegetables are likely to be due to a combination of substances acting simultaneously. The significant and positive correlations between glucosinolates and α- and γ-tocopherol reported in this study indicate added health benefit from consuming crucifer vegetables rich in these compounds.

Variation in glucosinolate levels between and within each group of *Brassica* vegetables suggests differences in their health-promoting properties. Recently, Faulkner et al. (1998) reported 10-fold higher glucoraphanin levels in hybrid lines developed from crosses between wild *Brassica oleracea* and broccoli accessions. When murine hepatoma Hepa 1c1c7 were treated with extracts from the high glucoraphanin lines, they exhibited more than a 100-fold increase in quinone reductase activity over broccoli accessions. Quinone reductase, glutathione *S*-reductase, and UDP-glucuronosyl transferase are phase II detoxification enzymes that have been shown to be induced by a diet rich in crucifer vegetables (Prochaska et al. 1992). These enzymes, through modification of the reactive products of cytochrome P450 oxidation, cause detoxification of carcinogens (Prochaska et al., 1992). Staack et al. (1998) reported that a mixture of glucosinolate breakdown products, similar to that found in a crucifer diet, greatly enhanced the synthesis of detoxification enzymes. They also found that glucosinolates interacted synergistically to induce higher levels of phase II detoxification enzymes and that the induction is dose dependent.

The diversity in glucosinolate levels reported in this and other studies suggests that the potential health benefits from crucifer vegetables are greatly dependent on the accession selected. Previous studies have suggested that enhancing the level of glucosinolates in cruciferous vegetables through conventional breeding or genetic engineering can be expected to enhance the chemopreventive properties of these vegetables. The wide range of variability in glucosinolates among and within the five *Brassica* groups that were evaluated in this study offer an important information base for developing accessions with enhanced health benefits.

NOMENCLATURE

Sinigrin, allyl-glucosinolate; gluconapin, butyl-3-enyl-glucosinolate; glucobrassicincanapin, pent-4-enyl-glucosinolate; progoitrin, (2*R*)-2-hydroxylbut-3-enylglucosinolate; epiprogoitrin, (2*S*)-2-hydroxylbut-3-enylglucosinolate; glucoiberin, 3-methylsulfinylpropyl-glucosinolate; glucoraphanin, 4-methylsulfinylbutyl-glucosinolate; glucoalysin, 5-methylsulpinlypentyl-glucosinolate; gluconaturtiin, phenethyl-glucosinolate; glucobrassicin, indol-3-ylmethyl-glucosinolate; neoglucobrassicin, *N*-methoxyindol-3-ylmethyl-glucosinolate; 4-OH glucobrassicin, 4-hydroxyindol-3-ylmethyl-glucosinolate; 4-CH_3O glucobrassicin, 4-methoxyindol-3-ylmethyl-glucosinolate; sulforaphane, 1-isothiocyanate-4-(methylsulfinyl)-butane.

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